

Amendments to the Specification

Please replace the section headings beginning at page 1, line 1, with the following amended section heading:

DESCRIPTION DESCRIPTION

Please replace the section headings beginning at page 1, line 5, with the following amended section heading:

TECHNICAL FIELD TECHNICAL FIELD

Please replace the section heading beginning at page 1, line 16, with the following amended section heading:

BACKGROUND ART BACKGROUND ART

Please replace the paragraph beginning at page 1, line 18, with the following amended paragraph:

α,α -Trehalose is a non-reducing disaccharide where two glucose molecules are bound via the α,α -1,1 glucosidic linkage. Although the amount of the saccharide is low, the saccharide extensively exists in nature, for example, in fungi, yeasts, bacteria, mushrooms, higher plants, insects, and the like. Because of its non-reducibility, α,α -trehalose causes no Maillard reaction (aminocarbonyl reaction) with substances having amino groups such as amino acids and

proteins. The saccharide causes no deterioration of ~~substance~~
substances comprising amino ~~acid~~acids, and is a stable
saccharide itself. Therefore, α,α -trehalose can be used and
processed without fear of browning and deteriorating and has
been expected ~~its uses in~~ to be useful in various fields.
~~Researches about~~ Research regarding the functions of α,α -
trehalose are now being in progress. However, under these
circumstances, ~~researches about~~ saccharide-derivatives of α,α -
trehalose, as saccharides which can be expected to have novel
functions not exhibited by α,α -trehalose, have been actively
performed.

Please replace the paragraph beginning at page 3,
line 25, with the following amended paragraph:

With regard to oligosaccharides having α -1,3
glucosidic linkage within their molecules, nigerose, a
reducing disaccharide where two glucose molecules are bound
via the α -1,3 glucosidic linkage, and nigerooligosaccharides,
having a structure of binding glucose with glucose residue at
the non-reducing ends of maltooligosaccharides *via* the α -1,3
glucosidic linkage, have been known (see Japanese Patent Kokai
Nos. 59,559/95 and 299,095/97). As disclosed in Japanese
Patent Kokai No. 52,834/97, it is known that saccharides

having nigerose as a structural unit have a strong immune-activating effect as well as ~~functions as sweetener such as~~ mild sweetness and taste-improving effect. However, since both nigerose and nigerooligosaccharide ~~have reducibilities~~are reducible, they have the disadvantages of easily causing the browning reaction with amino acids and causing the deterioration in the processing of foods.

Please replace the section headings beginning at page 4, line 21, with the following amended section heading:

DISCLOSURE OF INVENTION DISCLOSURE OF INVENTION

Please replace the paragraph beginning at page 5, line 1, with the following amended paragraph:

The present inventors have ~~been~~ extensively studied ~~en~~ novel saccharides, 3- α -glycosyl α,α -trehaloses and their preparation, to attain the above object. As a result, the present inventors have found novel saccharides, 3- α -isomaltosyl α,α -trehalose represented by the chemical formula 2 and 3- α -glucosyl α,α -trehalose represented by the chemical formula 3. The present inventors have also found that various other 3- α -glycosyl α,α -trehaloses can be easily synthesized by transferring other saccharides to these novel saccharides. The present inventors accomplished the present invention by

establishing saccharides comprising 3- α -glycosyl α,α -trehalose and processes for producing them. In addition, the present inventors accomplished the present invention by establishing compositions such as foods and beverages, cosmetics, and pharmaceuticals, comprising these saccharides or saccharide compositions comprising them.

Please replace the paragraph beginning at page 5, line 22, with the following amended paragraph:

3- α -Glycosyl α,α -trehaloses of the present invention are novel saccharides that have never been ever-unknownknown. Since the saccharides have both α,α -trehalose structure and nigerose structure within their molecules, the saccharide is expected to have various functions. Further, the present invention, which provides 3- α -glycosyl α,α -trehaloses, their preparation and use, is a useful invention that greatly contributes to this art.

Please replace the section headings beginning at page 6, line 3, with the following amended section heading:

BRIEF DESCRIPTION OF DRAWINGS BRIEF DESCRIPTION OF DRAWINGS

Please replace the section headings beginning at page 6, line 22, with the following amended section heading:

~~BEST MODE FOR CARRYING OUT THE INVENTION~~

BEST MODE FOR CARRYING OUT THE INVENTION

Please replace the paragraph beginning at page 7, line 2, with the following amended paragraph:

3- α -Glycosyl α,α -trehaloses of the present invention are not restricted by their origin ~~and~~ anor process for producing them as far as they have a 3- α -glucosyl α,α -trehalose structure represented by the aforesaid chemical formula 1 within their molecules. If they exist in nature, they can be also used in the present invention. Also, saccharides synthesized by chemical or enzymatic methods can be arbitrarily used. Both 3- α -isomaltosyl α,α -trehalose represented by the chemical formula 2 (abbreviated as "3- α -isomaltosyl α,α -trehalose", hereinafter) and 3- α -glucosyl α,α -trehalose represented by the chemical formula 3 (abbreviated as "3- α -glucosyl α,α -trehalose", hereinafter) are concrete examples included in the aforesaid 3- α -glycosyl α,α -trehaloses.

Please replace the paragraph beginning at page 8, line 4, with the following amended paragraph:

In the present invention, commercially available α,α -trehaloses are preferably used. As a commercially available α,α -trehalose, "TREHA[®]", a high purity hydrous crystalline α,α -trehalose product with ~~a~~an α,α -trehalose content of 98% or higher, commercialized by Hayashibara Shoji Inc., Okayama, Japan, can be advantageously used. Optionally, α,α -trehalose prepared by conventional methods, for example, by extracting from yeasts, collecting from bacterial culture ~~of bacteria~~ having α,α -trehalose-producing abilities, and allowing enzymes to act on starchy substances to form α,α -trehalose, can be advantageously used. A commercially available reagent grade panose, commercialized by Hayashibara Biochemical Laboratories Inc., Okayama, Japan, can be used as an α -isomaltosylglucosaccharide in the present invention. Optionally, panose can be prepared by conventional methods, for example, by hydrolyzing pullulan, a natural polysaccharide, by panose-forming α -amylase originated from a microorganism such as *Thermoactinomyces vulgaris*. Further, α -isomaltosylglucosaccharides such as panose, 4- α -isomaltosyl maltose, 4- α -isomaltosyl maltotriose, and the like can be prepared by allowing α -glucosidase capable of converting α -1,4

glucosidic linkage into α -1,6 glucosidic linkage and originated from microorganisms such as *Aspergillus niger*, *Aspergillus saitoi*, *Mucor javanicus*, *Penicillium crysogenum*, *Candida tropicalis*, etc. Furthermore, α -isomaltosylglucosaccharides prepared by α -isomaltosylglucosaccharide-forming enzyme originated from microorganisms such as *Bacillus globisporus* C9 (FERM BP-7143), *Bacillus globisporus* C11 (FERM BP-7144), *Bacillus globisporus* N75 (FERM BP-7591), and *Arthrobacter globiformis* A19 (FERM BP-7590), which are disclosed in International Patent Publication No. WO 02/055708 A1 applied for by the same applicant as the present invention, can be advantageously used.

Please replace the paragraph beginning at page 10, line 2, with the following amended paragraph:

Although 3- α -glucosyl α,α -trehalose can be chemically synthesized, the saccharide ~~can~~can be easily produced by allowing glucoamylase (EC 3.2.1.3) to act on a solution containing 3- α -isomaltosyl α,α -trehalose, which is obtained above, to specifically hydrolyze the α -1,6 glucosidic linkage of 3- α -isomaltosyl α,α -trehalose.

Please replace the paragraph beginning at page 14, line 5, with the following amended paragraph:

Since the sweetness of the saccharide composition comprising 3- α -glycosyl α,α -trehaloses and the high 3- α -glycosyl α,α -trehaloses ~~content~~ containing products, obtainable by purifying the saccharide composition, of the present invention well harmonizes with other tastes such as sourness, salty taste, astringency, delicious taste, and bitterness and they have satisfactory acid resistance and thermal stability, they can be advantageously used for sweetening, taste-improving, and quality-improving general foods and beverages.

Please replace the paragraph beginning at page 16, line 18, with the following amended paragraph:

When used as a quality-improving agent or stabilizer, they can be advantageously used in biologically active substances susceptible to ~~lose~~ losing their effective components and activities, as well as in health foods and pharmaceuticals containing the biologically active substances. Examples of such biologically active substances are liquid preparations containing lymphokines such as α -, β -, and γ -interferons, tumor necrosis factor- α (TNF- α), tumor necrosis factor- β (TNF- β), macrophage migration inhibitory factor,

colony-stimulating factor, transfer factor, and interleukin 2; liquid preparations containing hormone such as insulin, growth hormone, prolactin, erythropoietin, follicle-stimulating hormone, and placenta hormone; biological preparations such as BCG vaccine, Japanese encephalitis vaccine, measles vaccine, live polio vaccine, small pox vaccine, tetanus toxoid, *Trimeresurus* antitoxin, and human immunoglobulin; liquid preparations containing antibiotics such as penicillin, erythromycin, chloramphenicol, tetracycline, ~~streptomycin~~streptomycin, and kanamycin sulfate; liquid preparations containing vitamins such as thiamin, riboflavin, L-ascorbic acid, cod liver oil, ~~carotene~~carotenoids, ergosterol, tocopherol; solutions of enzymes such as lipase, elastase, urokinase, protease, β -amylase, isoamylase, glucanase, and lactase; extracts such as ginseng extract, turtle extract, chlorella extract, aloe extract, and propolis extract; living microorganisms such as virus, lactic acid bacteria, and yeast; and royal jelly. These biologically active substances can be easily prepared into health foods and pharmaceuticals, which have a satisfactorily-high stability and quality with less fear of losing or inactivating their effective components and activities by using them.

Please replace the paragraph beginning at page 17, line 16, with the following amended paragraph:

The methods for incorporating a saccharide composition comprising a large percentage of 3- α -glycosyl α,α -trehalose or a 3- α -glycosyl α,α -trehalose ~~high content product~~, obtained from the same, into the aforesaid compositions are those which can incorporate them into a variety of compositions before completion of their processing, and which can be appropriately selected from among the following conventional methods; mixing, dissolving, melting, soaking, penetrating, dispersing, applying, coating, spraying, injecting, crystallizing, and solidifying. The amount of them to be preferably incorporated into the final compositions is usually an amount of 0.1 % or higher, desirably, 0.5% or higher.

Please replace the paragraph beginning at page 38, line 21, with the following amended paragraph:

A starch suspension containing about 12.5% of "TREHA®", α,α -trehalose commercialized by Hayashibara Shoji Inc., Okayama, Japan, and about 12.5% of tapioca starch was prepared and admixed with 0.2%/g-starch of "NEOSPITASE", an α -amylase product commercialized by Nagase & Co., Ltd, Osaka, Japan, and then followed by the enzyme reaction at 85 to 90°C

for about 20 min. Subsequently, the starch solution was autoclaved at 120°C for 20 min and rapidly cooled to about 35°C and a liquefied starch solution comprising α,α -trehalose with a DE of about 2 was obtained. The liquefied starch solution was admixed with 0.3 ml/g-starch of the above concentrated enzyme solution and followed by the enzyme reaction at pH 6.0 and 35°C for 48 hours. After keeping the resulting reaction mixture at 95°C for 30 min, the reaction mixture was cooled and ~~filtrated~~filtered. According to conventional methods, the resulting filtrate was purified by decoloring with activated charcoal and desalting with H- and OH-forms of ion exchange resins. Further, the resulting saccharide solution was concentrated to give a concentration of 70% and then, a syrup comprising 3- α -isomaltosyl α,α -trehalose was obtained in a yield of about 90%, d.s.b.

Please replace the paragraph beginning at page 42, line 19, with the following amended paragraph:

Three parts by weight of gum base was melted by heating to give a soft texture, admixed with two parts by weight of anhydrous crystalline maltitol, two parts by weight of xylitol, three parts by weight of a syrupy saccharide product comprising 3- α -glucosyl α,α -trehalose, obtained by the method in Example 4, and appropriate amounts of flavor and

coloring, kneaded using a roll machine in a usual manner, shaped, and packed to obtain the chewing gum. The product has a satisfactory texture, taste, and flavor, and is suitable as a low calerie chewing gum with a low cariogenicity ~~and low~~ ~~calerie~~.

Please replace the paragraph beginning at page 45, line 18, with the following amended paragraph:

The product is a solid preparation for ~~fluid-a~~ liquid diet with a satisfactory stability. A bag of the product can be advantageously used for supplying energy to a living body by dissolving into about 150 to 300 ml of water to produce a fluid diet ~~and by using~~ that can be administered orally or by tube feeding to the nose, stomach, or intestines.

Please replace the section headings beginning at page 47, line 12, with the following amended section heading:

~~INDUSTRIAL APPLICABILITY~~ INDUSTRIAL APPLICABILITY

Please replace the paragraph beginning at page 45, line 18, with the following amended paragraph:

The product is a solid preparation for ~~fluid~~a liquid diet with a satisfactory stability. A bag of the product can be advantageously used for supplying energy to a living body by dissolving into about 150 to 300 ml of water to produce a fluid diet ~~and that can be administered by using~~ orally or by tube feeding to the nose, stomach, or intestines.